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CANINE TRACHEAL MUCOUS TRANSPORT OF NATURALLY-OCCURRING PARTICULATE POLLUTANTS

by

© Tzu Kuang Lee M.B., B.S.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Canine Tracheal Mucous Transport of Naturally-Occurring Particulate Pollutants submitted by Tzu Kuang Lee in partial fulfillment of the requirements for the degree of Master of Science in Experimental Medicine in the Department of Medicine.

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Supervisor

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.....

.....
External Examiner

Date June 19, 1979

DEDICATION

This thesis is dedicated to all my canine friends.

ABSTRACT

The study of the effect of the differences in physicochemical properties of different naturally-occurring particulate pollutants on their mucociliary clearance is made difficult by the inherent variability of measurements of mucous transport rates. Canine tracheal mucous transport rates (TTR) measured with 6 different markers (230 measurements) were analyzed in 109 healthy, nose-breathing, pentobarbital-anesthetized animals to examine its variability. Mean TTR, as measured by external tracking of I^{125} or Tc^{99m} labeled Dowex^{R} anion exchange resin particles, was 10.4 mm/min (n = 128, 40 dogs) and ranged from 1.8 to 25.8 mm/min with a coefficient of variation of 51%. Variability of TTR was similar for all types of markers analyzed, and similar between different dogs as within individual dogs. No difference between short-term (one experimental period up to 120 mins) and long-term (3 months) variation was found. Because of this great variability, a method that simultaneously tracked 2 different isotope-labeled markers was used to study the differences in TTR between 3 particulate pollutants, ragweed pollen (P) (an organic pollutant), and talc (T) and asbestos (A) (inorganic pollutants). The pollutants were labeled with Tc^{99m} using sodium pertechnetate and stannous chloride in HCl, and their respective TTR measured simultaneously against TTR of a reference marker, I^{125} labeled Dowex^{R} anion exchange resin particles (L) (12 dogs/37 experiments). As measurements were performed under the same laboratory conditions and physiological conditions of the animal, direct comparisons of TTR were obtained. No difference was found between TTR of T or A to L. However, P was faster in 13/22, equal in 5/22 and slower in 4/22 comparisons ($p < 0.025$) and was 19.3 ± 9.2 mm/min (mean $\pm$ SD) vs 16.7 ± 6.7 mm/min for

Abstract - continued:

L ($p < 0.05$). P appears to be transported faster than L, and faster than T or A by inference. This is consistent with the hypothesis that differences in the physicochemical properties of particulate pollutants may influence their interaction with the mucociliary system and result in differences in their mucous transport rates.

PREFACE

Polvere fa danno.

- Leonardo da Vinci

Some five hundred years ago, Leonardo da Vinci noted lung pathology in one of his dissections. He deduced that inspired air could carry dust into the lungs. In a drawing of this dissection, he wrote in his famed mirror writing the above notation, "dust causes damage" (94).

Today, our concern for the problems of dust and environmental pollution in general has grown in step with our knowledge of the diseases they cause. Perhaps the most remarkable biologic barrier that we possess against the environment is that which resides in the respiratory system. The normal adult breathes one thousand to two thousand liters of air a day, presenting his lungs with a variety of agents present in the atmosphere. These agents may belong to one of two categories, the non-viable and the viable. Non-viable agents include gaseous and particulate pollutants, organic and mineral dusts, radioactive agents and ions; viable agents include bacteria, viruses, algae, molds, yeasts, fungii, and spores. Biologic defenses generally comprise physical and humoral mechanisms, and lung defense is also considered along these lines. The mucociliary apparatus is a unique physical defense mechanism of the lung. Its importance in lung defense was appreciated by Hilding who wrote in 1943 (68):

> Death may result from physiologic failure of any one of a number of systems, such as the urinary, circulatory, or central nervous systems. Death may also occur from failure of the ciliary system

> within the respiratory tract, a fact which has apparently received little, if any, attention. In certain diseases of the lower respiratory tract, the cilia become destroyed and ciliary function is lost. Secretions collect, as a result, in such large quantities that the patients die of asphyxia.

The mucociliary apparatus is an integrated system involving the interaction of ciliary motility and the physical properties of the mucus that covers the ciliated surfaces. The respiratory tree, with the exception of few areas, is covered by ciliated epithelium from the upper airways to the terminal bronchioles. Inhaled particulate matter settling on these surfaces is transported by ciliary activity towards the pharynx, where it is swallowed.

This thesis attempts to add to the understanding of the mucociliary system. In particular it examines the transport of naturally-occurring particulate pollutants by the mucociliary apparatus.

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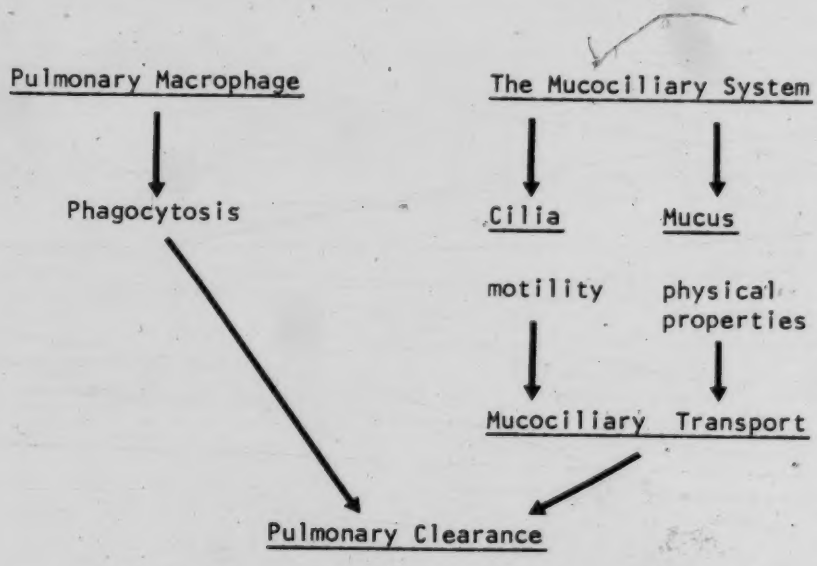
CHAPTER I INTRODUCTION

1. Introducing the Hypothesis

a. Background to the Problem

Mucociliary transport occurs from the interaction of ciliary activity with the physical properties of mucus and functions to remove particulate matter from the surface of the respiratory tract. The mucociliary system and pulmonary macrophages play the major roles in the overall clearance of particulate matter from the lung. Their interrelationship can be summarized (Rylander, 1966) (137):



The mucociliary system has been studied both *in vivo* and *in vitro*. Although cilia (133, 149, 152) and native mucus that covers ciliated epithelium (17, 155, 165) have been individually investigated, the integrated functioning of both entities is best examined in the study of mucus transport and mucociliary clearance. Mucous transport is studied in defined anatomical sites, while mucociliary clearance is studied through tracheobronchial clearance of inhaled particles (169).

Mucous transport has been studied by observing the movement of natural debris present in mucus (such as desquamated cells and air bubbles) (40, 74), or the placing of various markers on the mucus surface, and measuring their rate of transport by ciliated tissue. In vivo studies have been carried out in the trachea of chicks (10), rats (40, 54), cats (15, 30, 57, 90), donkey (5), sheep (89) and human nasal mucosa (128, 143) and trachea (51, 112, 178). Markers used include lamp black and lycopodium spores (30, 57, 90), India ink (9, 67), barium sulphate (159), pertechnetate solutions (53, 102), radiolabeled resin beads (142) and albumin microspheres (32, 178) and macroaggregates (174), and small teflon discs (51, 140).

b. The Variability of Mucous Transport

The canine trachea has been extensively used for the in vivo study of mucous transport (9, 32, 53, 67, 102, 140, 142, 170). In common with mucous transport rates studied in other animals and man (169, 178), canine tracheal mucous transport rates tend to vary greatly. Various investigators have alluded to this variability (36, 140, 142, 153, 178). Different investigators using similar techniques have obtained different values of canine tracheal transport rates (54, 102). Some of the reasons for this variation in measurements include the inherent variability of mucous transport rates within the animal itself (19, 36), fluctuations in degree of anesthesia (20,49, 89, 119) and level of hydration (33), and irritation of the tracheal mucosa as a result of experimentation (120) and changes in ambient laboratory conditions (142). The result is that the tracheal transport rate in an anesthetized dog can vary up to 200% in a single 30 minute experimental period (36).

This variability has raised problems in interpreting experiments on canine tracheal mucous transport. For example, the effects of a drug (52, 147, 178) or of physical therapy (33) on mucous transport cannot be interpreted unless there is a marked change in transport rates observed before and after intervention. Attempts to minimize this have included interpretations of the peak rates only (32, 33), or the average of a number of peak rates (51, 140).

In order to determine if the physiocochemical properties of a marker may influence its transport rate by the mucociliary system, a method which measures canine tracheal mucous transport rates of two different types of radio-labeled markers simultaneously in an animal has been developed (36). As measurements are performed within the same laboratory conditions and physiological conditions of the animal, direct comparison of transport rates of two types of markers are obtained. This has enabled study of the differences in transport rates between any of several types of different markers with differing physicochemical properties (36, 91).

c. The Influence of a Marker on Its Rate of Mucous Transport

Canine tracheal mucous transport rates similarly vary between different laboratories. In studies performed on anesthetized dogs, Asmendsson and Kilburn (9) using India ink as a marker obtained a mean tracheal mucous transport rate of 12.6 mm/minute, while Marin and Morrow (102) using drops of radioactive pertechnetate obtained a mean rate of 16 mm/minute. Similarly Sakakura and Proctor (142) using technetium-99m labeled resin beads, and Sackner et al (140) using small teflon disc markers, obtained mean tracheal mucous transport rates of

10.5 and 15.6 mm/minute, respectively.

Among the reasons accounting for these differences in observations may be the nature of the marker used to measure transport rates. Different markers possess different physicochemical properties (e.g. density, solubility, surface charge and chemical surface residues that interact with mucus), and may therefore interact differently with the mucus and/or cilia of the mucociliary system. Such interaction may influence their rate of mucociliary transport and has been the subject of recent studies (36, 91).

Inhaled naturally-occurring particulate pollutants possess different physicochemical properties from those of experimental markers. The study of mucous transport using the latter would not necessarily represent the situation in which an inhaled pollutant is transported by the mucociliary system. Measurement of mucous transport of the individual particulate pollutants themselves is therefore of greater relevance.

Moreover, particulate pollutants differ among themselves in their physicochemical properties. This may similarly affect their individual rates of transport by the mucociliary system. A particulate pollutant that is cleared by the mucociliary apparatus at a slower rate than another therefore resides for a longer period within the respiratory tree. The result may be reflected, for example, by a greater propensity for that pollutant to cause disease. This may partly explain the differences in the disease patterns caused by different pollutants in the lung (176). Comparison of mucous transport rates of different particulate pollutants is therefore of importance in the

understanding of the pathogenesis of pollutant-related lung disease.

2. Statement of Hypothesis

Naturally-occurring particulate pollutants that are inhaled are of different physical and chemical natures. These differences in their physicochemical properties may influence their interaction with the mucociliary system, and be reflected in differences in their mucous transport rates, measured *in vivo*.

3. Statement of Objectives

The objectives were to study the two interrelated aspects of canine tracheal mucous transport previously discussed viz its variability, and the transport of naturally occurring particulate pollutants, in the following way:

1. To document and analyze the variability of canine mucous transport rates, as measured using different types of markers.
2. To compare canine tracheal mucous transport rates of three selected radio-labeled naturally-occurring particulate pollutants, by the method of simultaneous measurement of marker transport rates in the same animal (36).

4. Justification of Study

Documentation and analysis of variability of mucous transport rates was performed to examine this aspect of mucous transport. It provided basic data that was useful within this study for subsequent

experiments on mucous transport and their interpretation, as well as for comparison with those obtained in other laboratories (140, 142). It also provided an insight into the problems involved in interpreting measurements of canine tracheal mucous transport rates, as well as to the reasons for adopting the method of simultaneous measurement of transport rates for the study of the particulate pollutants.

The method of simultaneous measurement of canine tracheal transport rates of two types of markers was used in studying transport of the pollutants as it provided direct comparison of their mucous transport, avoiding the factors of differing laboratory and animal conditions on the comparisons. As the retention time of a pollutant in the lung is partly determined by its mucociliary clearance and thus a factor in its causation of lung disease (176), comparison of mucus transport rates of different pollutants would be an important investigation in the study of diseases caused by inhaled pollutants.

CHAPTER II REVIEW OF THE LITERATURE

Three aspects of the literature will be reviewed: Inhalation and deposition of naturally-occurring pollutants, the structure and function of the mucociliary system, and the study of mucociliary transport.

1. Inhalation and Deposition of Naturally-occurring Pollutants

The constituents of the atmosphere differ regionally, depending on natural, industrial and occasionally therapeutic origins (48), and have been reviewed by Andersen (7). The atmosphere is an aerosol (113), i.e. a system of liquid droplets or solid particles dispersed in air, small enough to have a low settling velocity and thus capable of stability as an aerial suspension (65, 73).

Gaseous pollutants with a high water solubility (e.g. sulphur dioxide, ozone, ammonia) dissolve in fluid lining the mucosa and are mainly removed in the upper airways (108). Deposition of insoluble particulate pollutants in the respiratory tract depends on particle size (65, 71, 157) and respiratory patterns of the subject (65, 71, 115, 124). Inertial impaction occurs within the nose with particles greater than 10 to 15 microns in diameter. Gravitational sedimentation of particles 2 to 5 microns occurs in the more distal airways as air flow rates fall. Brownian diffusion occurs with particles 0.1 micron and smaller. Approximate size ranges for airborne inhalation hazards have been defined (157) and some examples of some airborne pollutants and their approximate size ranges are tabulated in Table I (data from Stuart (157) and Andersen (7).

TABLE I

APPROXIMATE SIZE RANGES OF SOME AIRBORNE POLLUTANTS *

Substance	Diameter (μm) Minimum	Maximum
Normal impurities in quiet outdoor air	0.01	1
Tobacco smoke	0.01	1
Metallurgical dust and fumes	0.001	50
Cement dust	3	100
Ground limestone	40	1000
Foundry dust	1	1000
Viruses and proteins	0.003	0.5
Bacteria	0.3	35
Plant spores	10	35
Pollens (various kinds)	10	100

* Data from Stuart (157) and Andersen (7).

Deposition of particles is further modified by their density, aerodynamic shape, hygroscopicity and electrical charge (71, 157) and by airflow velocity and aerodynamic characteristics of the airway, which become important especially in airway disease (56, 100, 158).

The fate of inhaled particles is varied (110). Some upper airway clearance occurs with blowing the nose (123) and coughing (93). The bulk of tracheobronchial clearance is however, by the mucociliary system (34, 61, 62, 114). Clearance of particles deposited beyond the reach of the mucociliary system in the rat occurs by alveolar-bronchiolar transport as demonstrated by Tucker (163). Green related this to energy derived from fluid transport along interstitial pathways and lymphatics (60). Morrow proposes that clearance of readily transportable material (e.g. dissolved or monomeric substances) may occur by both passive and active absorption processes, and less easily transportable material (e.g. insoluble dusts) by pinocytosis and dissolution (109). The pathogenesis of diseases associated with inhaled pollutants has been reviewed by Hunter (72) and Muir (113).

2. Structure and Function of the Mucociliary System

a. Respiratory Epithelium and Cilia

The structure of the respiratory airways has been reviewed by Proctor (125), Jeffery and Reid (77) and Breeze and Wheeldon (18). The nasal mucosa and most of the upper respiratory tract is lined by ciliated epithelium, interspersed with areas of non-ciliated columnar epithelium and areas of squamous metaplasia which are present even in normal airways. The larynx is lined by squamous epithelium except for

the posterior commissure which is lined with ciliated epithelium. Trachea and bronchii are lined by pseudostratified columnar epithelium consisting of ciliated and nonciliated columnar cells, goblet cells, basal cells, and intermediate cells, which may be precursors of either ciliated or goblet cells (92). Nonciliated columnar cells ("brush cells") with microcilli on their luminal surface or large microvilli on cell apices may represent developing or degenerating ciliated cells. These cells may have an absorptive function (76, 133). The major contributors to airway lining fluid are the goblet cells (17) and the submucosal glands (104) which increase in number in disease states such as chronic bronchitis (68, 77). Goblet cells extend from bronchial lumen to basement membrane, numbering 1 per 5 ciliated cells (133). They decrease in number from trachea to peripheral airways (92). Submucosal glands number about 1 per square millimeter of human airway surface and are 40 times greater in total volume than goblet cells. They tend to follow a similar distribution as goblet cells.

Cilia within the biological kingdom were first described by De Heide and Leuwenhouk in the seventeenth century. Investigation of their structure and physiology has been carried out by Satir (148, 149), comparative physiology by Sleigh (152), regulation and control of their movement by Kinosita and Murakami (85) and reviewed by Gosselin (59). Respiratory cilia were reviewed in a symposium in 1966 (159) and more recently by Sleigh (153). A ciliated columnar cell contains approximately 200 cilia which are 5-7 μm in length and 0.2 μm in diameter. Each cilium comprises two single fibrils forming a central core, surrounded by 9 fibrils with a doublet structure. The whole system is enclosed in a dense matrix. Each peripheral fibril, comprising 10 to

15 filaments of 1 nm diameter, join to form one structure towards the tip of the cilium. A basal body, with no direct connection with the fibrils, lies in the apex of each cell, corresponding to each cilium (148).

Cilia beat between 1,000 to 1,500 times a minute with a fast effective forward stroke 2 to 3 times faster than the slower recovery stroke. Ciliary bending is thought to be due to sliding of molecules (dynein arms) between the 2 subfibres of the peripheral elements, resulting in shortening of a doublet and bending of the cilium, with the basal body providing cyto-skeletal support (149). Energy for ciliary motion is derived from ATP, with ATPase activity being highly localized in peripheral fibrils (85). Intensity of ciliary motion is related to the level of cellular energy production (59). Congenital lack of dynein arms in spermatoza in 2 subjects with Kartagener's Syndrome (4) was associated with depressed mucociliary clearance (26), which is believed to be an important etiologic factor in the development of chronic airway disease associated with these patients (26, 45). Six parameters of ciliary motion are described, viz beat frequency, amplitude, force, direction, metachronal wave length, and wave velocity (59). Metachronism refers to the phenomenon of coordination of activity among cilia of adjacent cells resulting in interciliary movement generating a wave (152). It is described as symplectic, antiplectic or diaplectic, depending on whether the wave motion generated is in the same direction, opposite direction, or at a right angle respectively, to the direction of the effective stroke. Most preparations are antiplectic (86). In the airways, the effective stroke of ciliary motion is towards the oropharynx (153). Control of ciliary motion remains to be elucidated. Local

mechanical (159) and humoral factors such as serotonin, tyramine, GABA (59) and acetylcholine (38, 59, 87, 150) have been suggested. Nervous coordination of ciliary beat is not established and is not believed to exist in vertebrate and mammalian tracheas (20, 59, 80).

b. Airway Fluid

This has been reviewed recently by Lopez-Vidriero and Reid (96) and Keal (79), and in a textbook by Boyd (17). A variety of terms in the nomenclature, including "respiratory tract fluid" (Perry and Boyd, 1941) (17), "tracheobronchial secretions" (Yeager, 1971) (177) and "mucus" (Wardell, 1970) (172), have been used to describe the fluid that lines the airways and covers the cilia. In the absence of collective agreement so far (129), the term "mucus" will be used in this review synonymously with the other terms.

Mucus has several important functions. It is a protective barrier for airway mucosa against excessive dryness or harmful agents present in inspired air (17) and is also essential for mucociliary transport because of its rheologic properties (42, 81, 83, 95, 137, 141). In addition, it may stabilize peripheral airways against excessive changes in radius (99). The sources of mucus are multiple and difficult to define (79, 96). Submucosal glands and goblet cells are the major contributors. In addition, interstitial fluid particularly during inflammation (17), Clara cells, and alveolar type II pneumocytes, probably also contribute to fluid in the terminal bronchioles and alveoli (43). Mucus in the larger airways also receives contributions from peripheral airways because of constant mucociliary transport (67, 81). The total volume of airway mucus is estimated at between

0.2 to 0.3 ml/kg (Toremalm, 1960) (162) to 0.75 ml/kg body weight (Boyd, 1972) (17). There is convergence of a great surface area of distal airways on the smaller circumference of the trachea (67), and despite this and the ascension of more fluid by mucociliary transport, the depth of mucus (5 to 10 microns in larger airways) is preserved. It is suggested that some resorption must take place (81, 164).

Lucas and Douglas proposed in 1934 that fluid lining the respiratory tract is composed of 2 layers, a lower sol periciliary layer in which the cilia beat, and an upper gel mucous layer which interacts with the tips of cilia (97). Mathematical considerations suggest that the upper layer moves as an elastic slab (135), with a critical optimum depth for maximum velocity (13). Changes in the sol rather than gel layer might have greater influence on mucous transport (16). The upper layer may be coupled mechanically with flow induced in the serous layer resulting from ciliary beating (135). Despite extensive electron microscopy studies of the morphology of mucus <u>in vivo</u> by various investigators (Van As, 1974) (165), (Ebert, 1975) (44), (Sturgess, 1977) (158), (Yoneda, 1976) (179), the continuity of mucus remains incompletely defined (164). Submucosal glands or goblet cells, producing either acid or neutral glycoproteins, contribute to the upper layer, whilst the periciliary layer may represent alveolar or bronchiolar secretions (95) or may be derived from active water and solute transport across epithelium involving an active chloride pump (116).

Sade showed that mucociliary transport fails in the absence of mucus (141), though autologous or heterologous mucus can restore function (55, 141). King showed that this specificity of mucus was physical

(rheologic) rather than chemical (83). Rheologic studies on native tracheobronchial secretions are limited by difficulties of collection (41). Rheologic studies have been performed on sputum (2, 3, 42), surveying those from disease states (31, 121). Development of a method using plastic-coated fiberglass screens to collect native canine tracheal mucus by Proctor et al (1, 127) has further facilitated the rheologic (122) and biochemical (101, 130, 131) study of mucus. King has developed a method to study microsamples of mucus using a magnetic rheometer (84).

3. The Study of Mucociliary Transport

Mucociliary transport occurs from the interaction of ciliary motility and the rheological properties of mucus. It functions to remove particulate matter from the surface of the respiratory tract, an important factor in lung defense. Overall aspects of lung defense have been reviewed by Green (61, 62), Newhouse (114), Cohen and Gold (34) and by multiple authors in a series on Lung Biology in Health and Disease (132).

Methods used to study mucociliary transport have mainly belonged to one of two categories, viz methods examining tracheobronchial clearance of inhaled particles, and methods studying the transport of mucus, or markers deposited upon mucus, by ciliated epithelia. Wanner has distinguished these by the terms, "mucociliary clearance" and "mucous transport" study respectively (169). Studies have been performed in 3 main anatomical sites: the nasal passages, the trachea, and the airways of the lung (169).

a. Study of Mucociliary Clearance

This mainly studies tracheobronchial clearance and has been reviewed by Camner (23) and Wanner (169). Mucociliary transport is studied indirectly in this situation by external measurements of the retention of inhaled test aerosols of well-defined particles tagged with radionuclides. The first study carried out in humans (Albert and Arnett, 1955) (6) used aerosolized Fe^{59} particles. Sanchis et al have since performed similar studies using aerosolized saline suspensions of albumin labeled with Technetium-99m or Iodine-131 (144, 145), while Camner et al have developed 6 micron diameter polystyrene and teflon particles for similar studies (23,29). Mucociliary clearance studies have been performed in a variety of situations including the study of normal subjects (23, 29, 144), twins (28), cigarette smokers (27, 145), chronic obstructive lung disease (160), cystic fibrosis (146), after cholinergic and adrenergic stimulation (24, 25) and after lung denervation and bronchial transection (20).

The experimental factors influencing such studies include particle size in the inhaled aerosol and whether monodispersed or heterodispersed (111) particles are used (23), the type, number and location of scintillation detectors (23), respiratory rate and volume (65) and presence or absence of lung disease (56, 160). These factors determine the deposition pattern which affects clearance measurements, faster clearance rates being obtained from central zones than peripheral zones (112). Coughing during the experiment also affects measurements (93, 144). Sanchis et al (1972) using I^{131}-human serum albumin aerosol droplets found different mucociliary clearance curves in 3 zones in human lung, viz perihilar, intermediate, and peripheral.

The perihilar zone showed 3 phases of clearance, with half-lives of 1/2, 4 1/2 and 23 hours respectively; the intermediate, 2 phases with half-lives of 8 and 23 hours respectively; and the peripheral, 2 phases with half-lives of 1 and 23 hours respectively (144). These results are comparable with those of other investigators (6, 112). The experimental factors discussed above limit this mode of study of mucociliary transport. The slower phases of clearance probably represent non-mucociliary clearance mechanisms, involving physical solubility and alveolar macrophage activity, which cannot be distinguished from mucociliary activity (23, 169). Anatomic rather than regional mucociliary clearance has also been radiographically examined in airways after tantalum powder insufflation (46, 154, 175).

b. Study of Mucous Transport

Techniques used to study mucous transport have been reviewed by Asmendsson and Kilburn (8), Giordano and Morrow (54) and Wanner (169). A brief account of *in vivo* studies of mucous transport has been presented in Chapter I.

Methods to study mucous transport *in vitro* were reviewed by Rylander (51). *In vitro* methods often examine ciliary activity rather than mucous transport directly. Frog palates and esophagi and mammalian tracheas (e.g. rabbits, sheep, dog) have been used (169). The chief usefulness of *in vitro* studies has been in observing ciliary kinetics by stroboscopy (88), photography or photoelectricity (137), or in studying the effects of drugs (21, 47, 69, 75, 87, 150), or irritant aerosols (39, 40) on ciliary activity. Mucous transport on isolated frog palate is used in the rheological investigation of mucus, e.g. by King (83),

Giordano (55), Shih (151) and Dulfano (42). Corssen (1958) has used human respiratory ciliated explants cultured from punch biopsies to observe ciliary rotation (37, 38).

Methods to study mucous transport in vivo involve observation of cellular debris or air bubbles in mucus, or the placement of various markers on the surface of mucus and observing their rate of transport on the ciliated surface. The investigator (referenced), animal, marker, technique used, and in vivo mucous transport rate obtained in some studies on mammalian tracheas are summarized in Table II.

Mucous transport has also been studied under a variety of situations in vivo, including the study of transport rates at different levels in canine lung (9), in canine asthma (172), in cystic fibrosis (136, 174) and chronic obstructive lung disease in human subjects (147), after exposure of the tracheal epithelium to gases such as sulphur dioxide (40), cigarette smoke (170), nitrous oxide (54), oxygen (90, 102, 139), and aerosol hairspray (50). The effects of rhinovirus infection (143), nasal decongestants, ingested fluids and exercise (138) have been studied on nasal mucous transport. The effects on mucous transport of pharmacological agents (particularly adrenergic and cholinergic agents, biologically active amines, and methyl-xanthines) have been reviewed by Wanner (169) and Giordano (52), and will not be reviewed here. Experimental factors, including the use of anesthetic drugs, that influence mucous transport are discussed in Chapter V.

TABLE II

SOME METHODS EMPLOYED IN THE STUDY OF IN VIVO TRACHEAL MUCOUS TRANSPORT RATES IN MAMMALS

Synthesized and expanded from reviews by Giordano (54) and Wanner (169)

INVESTIGATOR	ANIMAL	MARKER	TECHNIQUE	MEAN TRANSPORT RATE (mm/min) OBTAINED
Dalhamn (40)	Rat	Cellular debris Air bubbles	G.A./microscopy (incised trachea)	13.5
Iravani (74)	Rat	Debris	G.A./microscopy	11
Giordano & Morrow (54)	Rat	Tc^{99m}pertechnetate	G.A./scintillation detector	8.1
Goldhamer & Carson (30)	Cat	Carbon-lyco-podium	G.A./microscopy (transillumination)	13
Laurenzi (90)	Cat	Carbon-lyco-podium	G.A./microscopy (transillumination)	22
Hilding (67)	Dog	India ink	NA/bronchoscopy	14-15*
Asmendsson & Kilburn (9)	Dog	India ink	G.A./microscopy (transillumination-intact & excised)	12.6
Giordano & Holsclaw (53)	Dog	Tc^{99m}pertech-netate	G.A./scintillation detector	10

Continued-

TABLE II continued:

INVESTIGATOR	ANIMAL	MARKER	TECHNIQUE	MEAN TRANSPORT RATE (mm/min) OBTAINED
Marin & Morrow (102)	Dog	Tc^{99m} pertechnetate	G.A./scintillation detector	16
Sakakura & Proctor (142)	Dog	Tc^{99m} resin beads	G.A./scintillation detector	10.5
Sackner (140)	Dog	Teflon discs	G.A./cinebronchofiberoscopy	15.6 **
Wanner et al (169)	Dog	Teflon discs	G.A./cinebronchofiberoscopy	13.5
Chopra (32)	Dog	Tc^{99m} / Indium ^{113m} } albumin microspheres	G.A./cinebronchofiberoscopy	19.2 **
Albert (5)	Donkey	Ferric-oxide-Cr^{51} polystyrene particles	G.A./scintillation detector	10 to 16
Landa (89)	Sheep	Teflon discs	NA/cinebronchofiberoscopy	17.5
Morrow (112)	Man	Ferric-oxide-Cr^{51} Mn^{54} O_2 polystyrene particles	NA/scintillation detector and NaI collimator	14

TABLE II continued:

INVESTIGATOR	ANIMAL	MARKER	TECHNIQUE	MEAN TRANSPORT RATE (mm/min) OBTAINED
Luchsinger (98)	Man	Cr^{51} polystyrene particles	NA/scintillation detector	T 1/2 ranged from 13 to 123 min (for trachea)
Friedman (51)	Man	Radio-opaque teflon discs	LA/radiography	10
Yeates (178)	Man	Tc^{99m} albumin microspheres	NA/scintillation detector	3.5

GA = general anesthesia

LA = local anesthesia

NA = no anesthesia

* = one dog studied only

** = average of maximum velocities measured

CHAPTER III MATERIALS AND METHOD

1. Selection of Animals

Healthy mongrel dogs were used in all studies. Animals of both sexes between 1 to 3 years of age and weighing between 12 to 35 kg were quarantined at the University of Alberta Dog Farm for a minimum period of 2 weeks. During this time, they were also vaccinated against diseases (distemper, hepatitis, and para-influenza), debarked, dewormed and deloused. Following quarantine, and in the absence of detectable disease, they were sent to the laboratory at the Surgical-Medical Research Institute, University of Alberta, for experimentation. Details and characteristics of the dogs used are listed in Appendix A and B.

2. Documentation of Variability of Canine Tracheal Mucous Transport Rates

a. Data Acquisition

All experiments measuring canine tracheal mucous transport rates (TTR) performed in Dr. S.F.P. Man's laboratory at the Surgical-Medical Research Institute, University of Alberta, from the period June, 1977 to January, 1979 were selected for analysis. Experiments where extreme changes occurred in the animal's physiological state, or where the effect of a drug or other intervention was studied, were not included in this analysis. Data from a total of 109 dogs (230 measurements of TTR) was analyzed.

The method of measurement of TTR included in the study of its variability, and the experimental conditions under which these were performed, were similar to those in the study on canine tracheal mucous

transport of particulate pollutants and are described under that section. There were two minor differences in methodology:

1. The use of a single pipette to deposit the markers in experiments prior to November, 1978 (36), instead of a pipette-rubber catheter combination used in the study of tracheal mucous transport of pollutants and,
2. the use of a scanning speed of 10 cm/min (36), instead of 20 cm/min in the latter study. Pilot experiments did not show significant differences in resolution between these two scanning speeds, though more data points could be obtained with the faster scanning speed.

b. Grouping, Analysis and Statistical Treatment

All TTR were grouped according to the type of marker used. Details of markers, physical characteristics, and radio-labeling used for each marker are listed in Table III.

(i) Overall Variability of TTR

The overall mean TTR, standard deviation and coefficient of variation (standard deviation ÷ mean x 100%) was calculated, and the highest and lowest TTR observed noted for each marker group. The mean TTR, standard deviation and coefficient of variation was also calculated for combinations of groups of markers having similar characteristics (e.g. all Dowex^{R} anion exchange resin particles). Stationary marker peaks were regarded as having zero TTR and not considered in any of the calculations.

TABLE III

MARKERS USED IN THE STUDY OF THE VARIABILITY OF CANINE TRACHEAL MUCOUS TRANSPORT RATES

MARKER		DIAMETER(microns)	RADIO-LABEL OF MARKER
Dowex^{R} anion exchange resin particles:			
(i)	Large (L)	180 ± 50	Iodine-125 or Technetium-99m
(ii)	Medium (M)	110 ± 30	Iodine-125 or Technetium-99m
(iii)	Small (S)	3 ± 3	Iodine-125 or Technetium-99m
Sulphur Colloid Suspension (SC)		0.2 - 0.5	Technetium-99m
Albumin Macroaggregates (MAA)		15 - 25	Technetium-99m
Dowex^{R} cation exchange resin particles*		180 ± 50	Gallium-67

* These are positively charged, hydrogen donors, as compared to negatively charged, chloride donors in the case of anion exchange resin particles.

(ii) Short-term Variability of TTR

Ten dogs with between 4 to 7 measurements each of TTR, measured with the same type of marker (I^{125}-labeled Dowex^{R} anion exchange resin particles of 180 $\pm$ 50μ diameter) over single experimental periods ranging from 30 to 120 minutes were selected for analysis of short-term variation of TTR. The mean TTR, standard deviation, coefficient of variation and range of TTR observed was calculated for each dog for each experimental period.

(iii) Long-term Variability of TTR

Results from 3 dogs, which were studied between 3 to 5 times each over a 3-month period using the same type of marker, were analysed for long-term variation of TTR. The mean TTR, standard deviation, coefficient of variation and range of TTR observed was calculated for each dog, over each of the 3-month periods.

3. Study of Canine Tracheal Mucous Transport of Particulate Pollutants

a. Selection of Animals and Type of Anesthesia

Similar animals to those previously described were used. Twelve healthy mongrel dogs of both sexes with weights ranging from 19 to 30 kg were fasted overnight before being anesthetized, usually between 0800 to 0900 hours on the morning of the experiments. Intravenous Pentobarbital 30 mg/kg body weight induction dose was used with supplemental doses as necessary, the aim being to maintain a relatively constant level of anesthesia. Hydration was maintained with 2/3-1/3 dextrose-saline infusion via peripheral vein. Vital signs were monitored

at intervals during the course of the experiments.

b. Selection of Particulate Pollutants

Three naturally-occurring particulate pollutants, one organic and two inorganic, were selected for study:

1. Whole ragweed pollen grains (Coulter Diagnostics, Inc., Hialeah, Florida, 33010), of spherical diameter 20 microns, specific gravity 1.20 - 1.50 (105) (organic pollutant).

2. Talc particles (Sierra talc), chemical formula $3MgO.4\ SiO_2\ H_2O$ of amorphous dimensions ranging from 2-25 microns, specific gravity 2.8 (64) (inorganic pollutant).

3. Asbestos particles (chrysotile form) (John-Manville Canada Ltd.), chemical formula $3MgO.2\ SiO_2.\ 2H_2O$ fibrillar in morphology with dimensions of 2.5 microns by 20-400 microns, specific gravity 2.0-2.8 (64) (inorganic pollutant).

c. Selection of a Reference Marker

Dowex^R anion exchange resin particles (1-X-2, 50-100 mesh) (Bio-Rad Laboratories, 2200 Wright Avenue, Richmond, California, 94804) were selected as the reference marker because of ease and high efficiency of labelling. These are styrene divinylbenzene copolymers, with diameter 180 $\pm$ 50 microns, specific gravity 1.15 (173).

d. Labeling of Reference Marker with Iodine-125

Approximately 100 mg of the particles were washed repeatedly with deionized water to remove any extraneous chemicals remaining from

their manufacturing process, and incubated with a solution of NaI^{125} (5 mCi) at room temperature for 30 minutes. They were then washed several times with deionized water to remove any free and loosely bound isotope, and the labeling efficiency as well as affinity of binding of isotope to marker noted. The average activity of isotope used per experiment was 200-400 μCi.

e. Labeling of Particulate Pollutants with Technetium-99m

All 3 pollutants were labeled in a similar fashion by a method adopted from Van Houten (166). Labeling was done immediately prior to experiments because of the short half-life (6 hr) of Tc^{99m}. Technetium, as it exists in the $^{99m}TcO_4^-$ state, is reduced to the ^{99m}Tc cation group, which then binds to nucleophilic groups present on the surface of the particle being labeled (166). Stannous chloride was used as the reducing agent, by dissolving 15 mgm of fresh crystals of this substance in 1.0 ml of 1 N HCl, and diluting the resulting solution to 100 ml. One ml of this solution was then combined with 1.0 ml of sodium pertechnetate solution (10-15 mCi) and a predetermined quantity of particulate pollutant. These were incubated at room temperature with gentle stirring by means of a magnetic stirring bar for 30 minutes, to ensure maximum exposure of the surfaces of individual particles to the isotope. The labeling reaction was terminated by adding a solution of IN NaOH dropwise until a pH of 7.0 was obtained. The labeled particles were then washed several times with deionized water, and harvested by centrifugation and finally resuspended in 0.6 ml of IN saline. This yielded sufficient quantity of labeled pollutant marker for 12 to 15 experiments. The average activity of each

bolus of labeled pollutant used per experiment was 200-600 μCi.

f. Assessment of Effects of Labeling Procedures on Reference Marker and Particulate Pollutants

The reference marker as well as each particulate pollutant were examined under light microscopy after undergoing the labeling procedure, to assess any alteration of morphology of the marker or pollutant that might occur. A hematocytometer counting chamber (Brightline counting chamber, American Optical Company, Buffalo 15, N.Y.) was used to estimate and confirm the dimensions of the individual markers and document their individual morphology. In a series of pilot experiments, each marker was submitted to the labeling procedure with substitution of normal saline for isotope, to avoid subsequent handling of radioactive material under the microscope. Each was then similarly re-examined under light microscopy.

g. Assessment of Adequate Activity of Labeled Particulate Pollutants for Experiments

The activity of the reference marker was found previously to be sufficient for detection and resolution by the scanning apparatus following the labeling procedure (36). Preliminary experiments were also done to determine this factor for each particulate pollutant. One drop of a suspension of freshly-labeled pollutant placed in a small well provided a 1 mm point source of activity. Two point sources at a maximum distance of 15 cm from the detector head of the scanner and separated by a minimum distance of 2 mm along the traveling axis of the scanner, could be satisfactorily discriminated as separate peaks of activity at a scanning speed of 20 cm/min. The distance of

the detector head from the trachea was generally under 10 cm during actual experimental conditions.

h. Device Design for Deposition of Marker/Pollutants

To minimize the number of times a pipette had to be passed across the vocal cords to deposit the markers, a device was designed to allow the reference marker and pollutant to be loaded and deposited separately on one instrumentation (Figure 1). Marker and pollutant were kept separate prior to deposition to minimize any possible marker-pollutant interaction. A plastic pipette tip with a soft, flexible rubber guard to minimize mechanical trauma to the tracheal epithelium was attached to a 30 cm glass pipette. A 15-G bore Venocath ^{R}-14 rubber catheter was attached to the length of the glass pipette with its tip alongside that of the plastic pipette tip. Approximately 2 mgm of Dowex^{R} anion exchange resin particles (reference marker) was placed in the pipette tip. The labeled particulate pollutant (approximately 2.0 mgm pollen, 4.0 mgm talc, or 1.0 mgm asbestos for each experiment) was drawn up as a suspension into the catheter in the form of several beads of suspension separated by gaps of air (Figure 1).

i. Procedure for Marker/Pollutant Deposition

The distance from carina to lower incisor teeth was pre-measured externally in each dog to note the approximate distance needed to advance the pipette. Care was taken not to touch the tracheal mucosa during insertion of the pipette to minimize the effects of mechanical irritation on TTR (120). The reference marker and pollutant were deposited separately, within several seconds of each other, on the posterior membranous portion of the trachea over the carina by

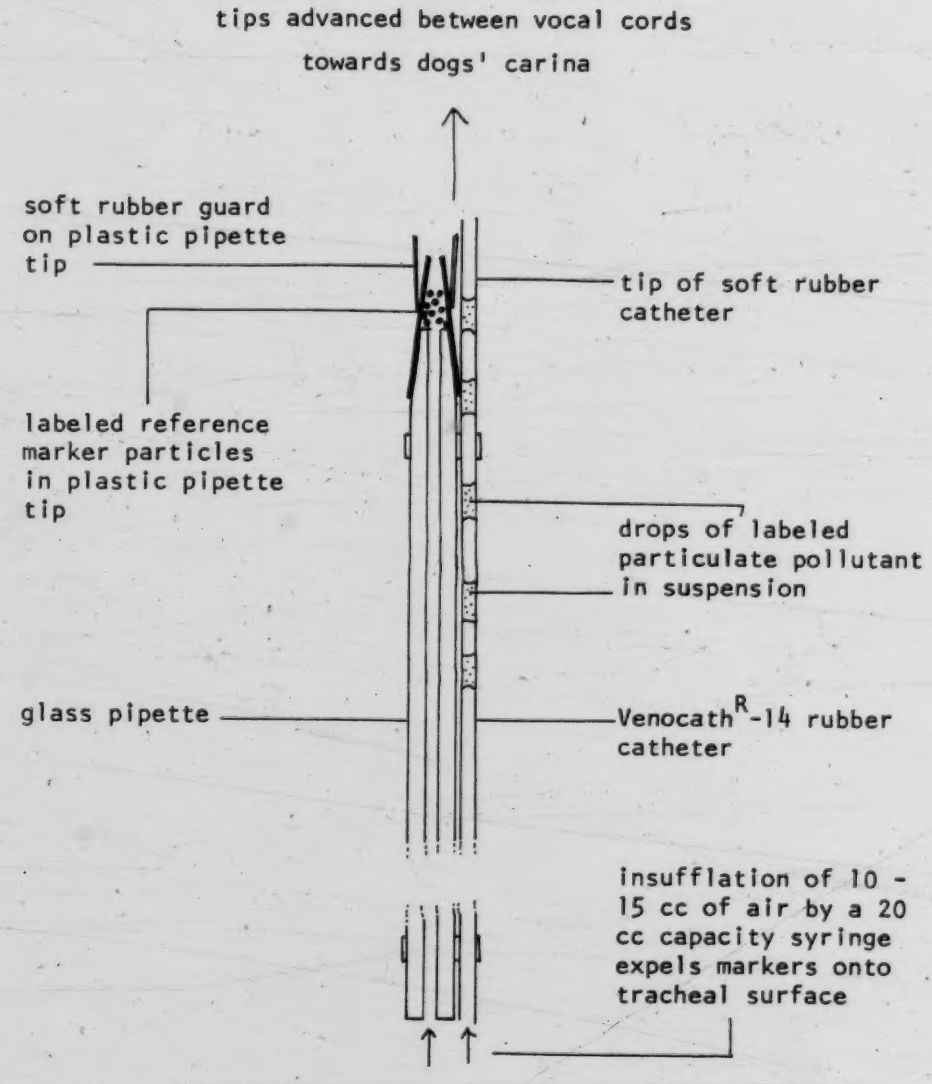

tips advanced between vocal cords
towards dogs' carina
soft rubber guard on plastic pipette tip
tip of soft rubber catheter
labeled reference marker particles in plastic pipette tip
drops of labeled particulate pollutant in suspension
glass pipette
Venocath R -14 rubber catheter
insufflation of 10 - 15 cc of air by a 20 cc capacity syringe expels markers onto tracheal surface

FIGURE 1

DIAGRAM OF DEVICE USED TO DEPOSIT RADIO-LABELED MARKERS INTO THE CANINE TRACHEA

insufflation of 10 to 15 ml of air through the pipette or through the rubber catheter. The order of deposition was alternated between experiments for randomization of deposition. If isotope movement was observed, scanning was begun and continued until no more peak movements were detected for 5-10 minutes. The procedure was repeated in some cases, with a maximum of 3 procedures performed on any one dog. The choice to repeat the procedure on any dog was at random. In the case where no isotope movement was detected following the first instrumentation, it was not repeated.

As deposition in these experiments was necessarily a "blind" procedure, preliminary studies were carried out to investigate the accuracy and pattern of deposition of markers at the carina using this device. In separate pilot experiments, a PHO gamma scintillation camera (Searle Radiographics, Des Plaines, Ill.) was used to monitor marker deposition and movement in 5 anesthetized dogs. Tc^{99m}-labeled sulphur colloid was placed in the rubber catheter and deposited in the manner previously described. Direct observation of marker deposition and movement by fiberoptic bronchoscopy was also carried out in one anesthetized dog, using orange-coloured Dowex^{R} resin particles and violet-coloured ink as markers.

Deposition of reference resin marker and ragweed pollen grains on the mucous layer was studied histologically in rat trachea. Unlabeled marker and pollen grains were injected into the trachea of a rat anesthetized with 0.2 mgm/kgm pentobarbital. The trachea was clamped and removed between glottis and carina after 1 minute to allow both markers time to distribute in the mucus, and immersed in

20% glutaraldehyde solution for 30 minutes. The preparation was transferred to formaldehyde, and paraffin sections with hematoxylin-eosin stains prepared in the usual way and examined under light microscopy.

j. Tracing and Measurement of Marker/Pollutant

All depositions and measurements of movement of marker and pollutant were performed with the animal in the supine position and spontaneously nosebreathing, and the long axis of the trachea in a horizontal plane. All measurements of TTR were performed between 0900 and 1800 hours on the day of the experiment, commencing an hour or longer after induction of anesthesia to allow the acute effects of anesthesia to dissipate.

Measurement of isotope movement was by the method developed previously in the laboratory by Man et al (36). External counting of a predetermined segment of trachea (10 to 11 cm) was performed using a Picker magnascanner 500D for periods ranging from 15 to 35 minutes, depending on number of isotope movements detected. The detector head comprised a collimated sodium iodide crystal traveling at a scanning speed of 20 cm/min along the long axis of the trachea. The collimator had a 1 mm slit, and the energy levels of 30 and 140 keV for Iodine-125 and Technetium-99m, respectively, allowed discrimination of the isotope to be tracked. This was done using a manually operated switch for the different energy levels. Appendix C further details these aspects.

The activity of one isotope was measured during movement of the detector head towards the glottis, and that of the other isotope during movements towards the carina. Activity and position measurements

were recorded on magnetic tape with a Northern Scientific NS 636 dual parameter multichannel analyzer with a 64 by 64 channel matrix interfaced to a PDP 11-05 minicomputer. The scanner was interfaced to the multichannel analyzer so that the distance traversed by the collimated detector corresponded to a maximum of 64 discrete channels. When the 64 channels were spread over a maximum scanning length of 14 cm, points 0.218 cm apart could be discriminated. A single experiment could also record up to 64 sequential traverses of the trachea. Marker or pollutant movement could therefore be inferred, and TTR calculated, by examining the activity distribution of several traverses. Only peaks of activity greater than 5 times that of background activity were analyzed, though the majority of peaks obtained were over 20 times background activity. From each experiment, distance versus time was plotted for each peak activity, detected in all the traverses where the same peak (same group of marker/pollutant particles) was present. Distance versus time was also plotted graphically, the slope being the TTR of the marker or pollutant. Marker and pollutant movement was found to be uniform, with an average regression coefficient of 0.98 for points obtained. The above procedure is summarized in Figure 2.

k. Analysis and Statistical Treatment

(i) If two different markers, i.e. the radio-labeled reference resin particle and the radio-labeled particle of pollutant being compared, were tracked and found to be moving at the same time over a similar segment of trachea, they were considered for comparison. They were usually followed together for periods ranging from 3 to 15 minutes

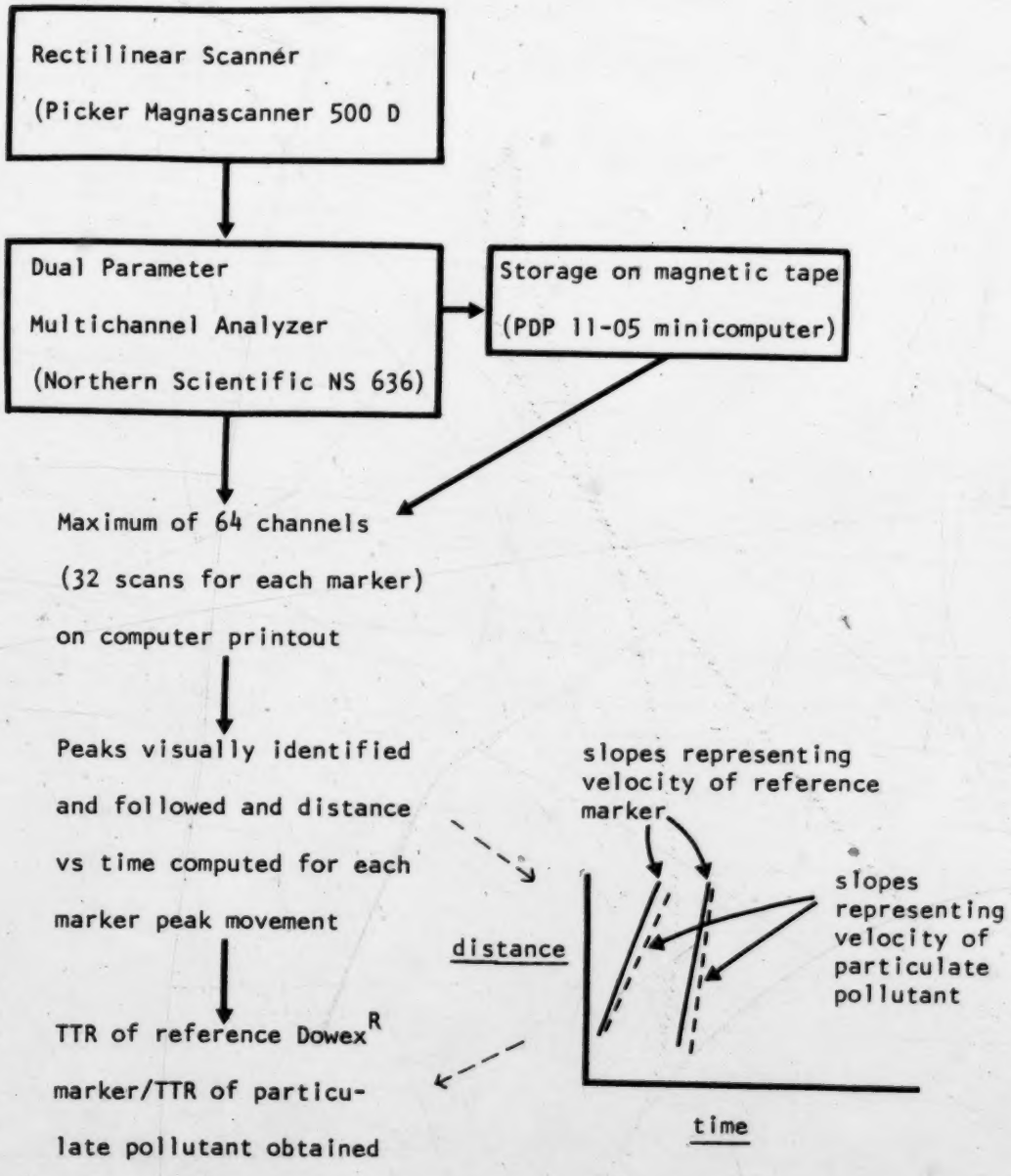


FIGURE 2

SUMMARY FLOW-DIAGRAM OF

MEASUREMENT OF MARKER MOVEMENT*

* Instruments denoted in boxes

on analysis. The individual TTR for each marker in a pair was calculated.

(ii) The TTR of pollutant was divided by the TTR of reference marker in each pair to give a ratio. Allowing for limits of error within the measurements of TTR, ratios between 0.95 - 1.05 were considered "ties"; greater than 1.05, "faster", and less than 0.95, "slower", with respect to TTR of pollutant vs reference marker.

(iii) The number of times pairs were tied, faster or slower with respect to movement of the pollutant particle was then treated with tests of proportion (35). Tracheal transport rates of peaks in each series of experiments were also treated with Student's paired t-test (35). In the case of more than one TTR obtained against another of the other marker, one value was picked using a table of random numbers to make a pair, and similarly included into the paired t-test.

CHAPTER IV RESULTS

1. Documentation of Variability of Canine Tracheal Mucous Transport Rate

a. Variability of Rates of Different Markers

The results are shown in Table IV.

Results from six different types of markers were analyzed. The largest number of TTR measured were from the group of markers comprising I^{125} or Tc^{99m}-labeled Dowex^{R} anion exchange resin particles of 180 $\pm$ 50 micron size ("large"), which involved 40 dogs/128 measurements, performed over 18 months. The TTR of this group showed a mean $\pm$ SD of 10.4 $\pm$ 5.3 mm/min, with a coefficient of variation of 51%. The range was from a low of 1.8 mm/min to a high of 25.8 mm/min, (approximately a 14-fold difference).

Essentially similar results were obtained from the analysis of measurements of TTR using "medium" and "small" I^{125} or Tc^{99m}-labeled Dowex^{R} anion exchange resin particles, Tc^{99m}-labeled sulphur colloid suspension, Tc^{99m}-labeled albumin macroaggregates, and Gallium-67 labeled cation exchange resin particles (Table IV). The coefficients of variation of TTR were similar in all these groups of markers used (about 50%) and each group showed a wide range in TTR observed (all over a 10-fold difference between lowest and highest TTR). A similar trend was seen on the combined groups of markers with similar characteristics analyzed together viz "medium" plus "small" combined, and "medium", "small", Tc^{99m}-sulphur colloid plus Tc^{99m}-albumin macroaggregates. (Ga^{67}-cation exchange resin did not fall into the characteristics of any group, and was not included in any of the

TABLE IV

CANINE TRACHEAL MUCOUS TRANSPORT RATES MEASURED USING SIX DIFFERENT TYPES OF MARKERS

Marker*	No. of dogs**/ no. of measurements	Tracheal Transport Rates: Mean ± SD (mm/min)	Coefficient of Variation (%)	Range (mm/min) Lowest	Highest
I^{125} or Tc^{99m} Dowex^{R} anion exchange resin particles					
Large (L)	40/128	10.4 ± 5.3	51	1.8	25.8
Medium (M)	6/13	9.3 ± 4.6	50	1.7	15.8
Small (S)	14/28	10.9 ± 5.4	49	2.2	23.9
Tc^{99m} sulphur colloid (SC)	21/3	9.7 ± 5.8	59	1.5	23.8
Tc^{99m} albumin macro-aggregates (MAA)	8/15	7.9 ± 4.7	59	1.8	18.0
Ga^{67}-Dowex^{R} cation	5/10	10.5 ± 3.9	37	5.5	17.5
Combined (M + S)	20/41	10.4 ± 5.2	50	-	-
Combined (M + S + SC + MAA)	40/92	9.7 ± 5.3	55	-	-

* for differences between markers, see Table III

** some dogs were used more than once, within the same group and among different groups of markers in the study

combined groups analyzed).

b. Variability of Rates in Single Experimental Periods in Different Dogs

The results are shown in Table V.

Ten dogs each had between 4 to 7 measurements of TTR performed over single experimental periods ranging from 30 to 120 minutes in each case. All measurements of TTR were made with I^{125}-Dowex^{R} anion exchange resin particles, 180 $\pm$ 50 micron size, as the marker. The coefficient of variation of TTR within an experimental period (short-term variation) for all the dogs studied ranged from 18% to 68%, with a mean of 41%.

The mean TTR of individual dogs ranged from 8.7 mm/min in dog 7 to 20.2 mm/min in dog 5. Taking the ten mean TTR for the group, the group mean $\pm$ SD of TTR was 13.8 $\pm$ 4.0 mm/min (coefficient of variation of 30% for the group).

The range of TTR in a single experimental period in each dog varied from between 14.6 - 25.0 mm/min in dog 5 (170%) to 2.9 - 15.7 mm/min (540%) in dog 7, with an average difference of 320% between the highest and lowest TTR obtained in any experimental period for all 10 dogs.

c. Variability of Rates in Different Experimental Periods in Single Dogs

The results are shown in Table VI.

TABLE V

CANINE TRACHEAL MUCOUS TRANSPORT RATES MEASURED IN SINGLE EXPERIMENTAL PERIODS* IN TEN DIFFERENT DOGS

Dog Number	Individual Rates (mm/min)	Tracheal Transport Rates: Mean ± SD (mm/min)	Coefficient of Variation (%)	Range (mm/min) Lowest	Highest
1	20.7, 12.4, 24.0, 13.9, 29.5 (n=5)	20.1 ± 7.1	35	12.4	29.5
2	9.2, 7.3, 21.3, 16.2 (n=4)	13.5 ± 6.5	48	7.3	21.3
3	10.4, 7.2, 7.0, 8.6, 15.2, 10.8, 17.8 (n=7)	11.0 ± 4.1	37	7.0	17.8
4	12.4, 10.1, 18.7 14.1, 4.4 (n=7)	11.9 ± 5.3	45	4.4	18.7
5	25.0, 17.9, 21.3, 18.8, 24.2, 14.6, 19.6 (n=7)	20.2 ± 3.6	18	14.6	25.0
6	7.1, 12.3, 16.3, 17.0 (n=4)	13.2 ± 4.5	34	7.1	17.0
7	11.3, 2.9, 4.8, 15.7 (n=4)	8.7 ± 5.9	68	2.9	15.7
8	17.6, 11.2, 9.3, 4.2 (n=4)	10.6 ± 5.5	52	4.2	17.6

Continued-

TABLE V continued:

Dog Number	Tracheal Transport Rates: Individual Rates (mm/min)	Mean ± SD (mm/min)	Coefficient of Variation (%)	Range (mm/min) Lowest	Highest
9	13.8, 13.9, 15.4, 10.1, 4.7 (n=6)	11.7 ± 3.9	33	4.7	15.4
10	21.0, 13.9, 20.5, 6.8, 22.6 (n=5)	17.0 ± 6.6	39	6.8	22.6

* All measurements of TTR were performed on the same day, within a 30-120 minute experimental period for each dog. I^{125}-Dowex^{R} anion exchange resin particles, 180 ± 50 micron size was the marker used in all measurements. This data was from a series of experiments where simultaneous transport of 2 markers was being studied; the other marker in all these experiments was Tc^{99m}-sulphur colloid. Variability of TTR of the latter marker (not shown here) was of the same degree.

TABLE VI

CANINE TRACHEAL MUCOUS TRANSPORT RATES MEASURED IN DIFFERENT EXPERIMENTAL PERIODS* IN THREE DOGS

Dog Number	Date of Study	Tracheal Transport Rates** (mm/min) Individual Rate(s)	Mean
1	21 Nov 1978	0	0
	11 Dec 1978	12.8	12.8
	15 Jan 1979	20.7, 12.4, 24.0, 13.9, 29.5	20.1
10	29 Nov 1978	10.1	10.1
	11 Dec 1978	6.2	6.2
	11 Jan 1979	9.2, 7.3, 21.3, 16.2	13.3
	30 Jan 1979	21.0, 13.9, 20.5, 6.8, 22.6	17.0
	8 Feb 1979	4.6, 5.4	5.0
11	14 Dec 1978	13.7	13.7
	11 Jan 1979	24.5	24.5
	30 Jan 1979	23.9, 32.6, 16.9	24.5
	8 Feb 1979	0	0

* All experimental periods ranged from 30-120 minutes, the experiments taking place over a 3-month period. I^{125}-Dowex^{R} anion exchange resin particles, 180 $\pm$ 50 micron size was the marker used in all measurements. This data was from a series of experiments where simultaneous transport of 2 markers was being studied; the other marker in all these experiments was Tc^{99m}-sulphur colloid.

** Zero tracheal transport rates (stationary marker activity peaks) recorded for sake of subsequent discussion.

Three dogs were studied from 3 to 5 times each over a 3-month period. All measurements of TTR were made with I^{125}-Dowex^{R} anion exchange resin particle, 180 ± 50 micron size, as the marker.

Dog 1 was studied 3 times (between November 1978 - January 1979), Dog 11, 4 times (between December 1978 - February 1979), and Dog 10, 5 times (between November 1978 - February 1979). The mean values of TTR differed considerably in each dog between all the occasions studied. Furthermore, dogs 1 and 11 showed no marker movement on one occasion each, though marker movement was observed on the other occasions they were studied.

Taking the TTR's obtained on each occasion for each dog (single values and means), the mean ± SD of TTR for Dog 1, 10 and 11 were 11.0 ± 10.1, 10.4 ± 5.0, and 15.7 ± 11.6 mm/min respectively, for each of their 3-month periods of study. This gave a coefficient of variation (long-term variation) of TTR of 92%, 48% and 74% respectively for the three dogs.

2. Study of Canine Tracheal Mucous Transport of Particulate Pollutants

a. Labeling of Reference Marker and Particulate Pollutants

The results are summarized in Table VII.

Labeling of the reference marker, Dowex^{R} anion exchange particles (1-X-2, 50-100 mesh) with Iodine-125 was highly efficient with a labeling efficiency of over 90%. This was calculated by dividing the radioactivity found on the labeled marker by the total activity of all the unbound isotope in the washes plus the activity on the tagged

TABLE VII

LABELING CHARACTERISTICS OF REFERENCE MARKER

AND PARTICULATE POLLUTANTS

Isotope Label / Marker or Pollutant	Quantity of Reference Marker or Pollutant Used (mg)	Percent Labeling Efficiency**	Percent isotope freed in last 3 washings ***
I^{125} / Reference Marker*	100	over 90	<1
Tc^{99m} / Pollen	30	15 - 20	<1
Tc^{99m} / Talc	60	60 - 77	<10
Tc^{99m} / Asbestos	15	32 - 91	<1

* Dowex^{R} anion exchange resin particles (1-X-2), 50-100 mesh).

** This is derived from final activity of labeled reference marker or pollutant ÷ by total activity of all the unbound isotope in the washes plus the activity of the labeled reference marker or pollutant. These values have been subsequently exceeded with improvement in technique (unpublished data).

*** This is derived from activity in each of the last 3 washings ÷ by the final activity of the labeled reference marker or pollutant.

marker. Less than 1% of isotope came free in the final 3 washings. Dissociation of the isotope from the particles in the airway was therefore unlikely.

Labeling efficiency of the particulate pollutants (whole ragweed pollen grains, talc and asbestos) with Technetium-99m was 15-20%, 60-77%, and 32-91%, respectively. In order to ensure enough activity on the tagged pollutant required for the experiments, pertechnetate solutions of at least 10-15 mCi of activity were always used, especially in the case of labeling of pollen. Dissociation of the isotope from the pollutant particles in the airway was also unlikely because of the small amounts of isotope freed in the last 3 washings (Table VII). It was known from previous experiments that free isotope in the trachea resulted in a "smear" pattern of activity detected on scanning. This was not found in any of the experiments.

Assessment of the reference marker and each pollutant by light microscopy (as described in Chapter III) revealed no discernible morphological alterations as the result of the labeling procedure.

b. Pilot Studies on Deposition of Markers

By use of a gamma scintillation camera, the deposition of Tc^{99m} sulphur colloid by the device designed for the experiments was tested. Deposition was observed to occur over a 4-5 cm region near the carina. The marker, either as a single bolus or as several boluses of activity, was observed to move towards the glottis at varying rates. Clearance of the Tc^{99m}-sulphur colloid marker from the trachea, as monitored by an external counter, exceeded 95%, over periods ranging from 15 minutes to an hour. In one animal, no movement of marker occurred when observed

over a period of an hour.

Direct observation by a fiberoptic bronchoscope of marker deposition was also carried out. To aid visualization, orange-coloured Dowex^{R} resin particles (approximately four times the size of the reference particle used in the experiments) and violet-coloured ink were used as markers. Deposition was seen to occur in the region of the carina, and occasionally beyond it. The particles and droplets of markers were found lying discretely apart from one another in most areas of the mucosa following deposition. Markers initially deposited on the side walls of the trachea tended to move towards the posterior membrane as they traveled towards the glottis.

The distribution of reference resin particles and ragweed pollen grains on the mucous layer of rat trachea was examined histologically. Both were distributed on the mucosal surface of the mucus. Little, if any, penetration into the mucous layer by either marker or pollen grain was observed. No studies were performed for talc or asbestos.

c. Results and Analysis of Simultaneous Measurements of Tracheal Mucous Transport Rates of Pollutants and Reference Marker

The results of tracheal mucous transport rates of reference marker and each pollutant and their comparisons are shown in Tables VIII to X, and the results of comparisons summarized in Table XI.

In comparing the tracheal transport of whole ragweed pollen grains to the reference marker, 13 experiments were performed on 6

TABLE VIII

CANINE TRACHEAL MUCOUS TRANSPORT RATES

OF REFERENCE MARKER vs RAGWEED POLLEN

Dog Number/Experiment Number		Tracheal Transport Rate (mm/min)		
		Reference Marker	Pollen	Pollen ÷ Reference Marker
12.	(1)	7.8*	* 7.1	0.91
			* 11.7	1.50
	(2)	15.2*	* 14.7	0.97, 2.04
		7.2*+	* 16.5	1.08, 2.29
		15.4+	+ 13.9	1.93, 0.90
13.	(1)	21.0*	* 23.4	1.11
		13.9+	+ 13.3	0.96
	(2)	20.5*	* 23.3	1.14
		6.8+	+ 6.9	1.01
	(3)	22.6*	* 31.6	1.40
			* 34.0	1.50
14.	(1)	23.9*	* 34.2	1.43
	(2)	32.6*	* 36.5	1:12
		16.9	-	-
15.	(1)	15.0*	* 13.2	0.88
	(2)	2.0	-	-
		6.5	-	-
16.	(1)	13.0*	* 10.5	0.81
	(2)	21.8*	* 19.8	0.91
		12.9+	+ 19.6	1.52
	(3)	22.0	-	-
		19.9*	* 22.7	1.14
		13.7+	+ 13.7	1.00
17.	(1)	no marker movement observed		

*,+ denote the tracheal transport rates within the experiment corresponding to a pair for comparison. These rates were paired if they were measured when the reference marker and pollen were moving simultaneously along a similar segment of trachea. In dog 15, experiment 2, only movement of reference marker was noted and no comparisons were obtained. In dog 17, no movement of either reference marker or pollen was noted, and the experiment was not repeated. The total number of comparisons obtained was 22.

TABLE IX

CANINE TRACHEAL MUCOUS TRANSPORT RATES

OF REFERENCE MARKER vs TALC

Dog Number/Experiment Number		Tracheal Transport Rate (mm/min) Reference Marker	Talc	Talc ÷ Reference Marker
12.	(1)	-	10.3	-
		-	4.6	-
	(2)	-	14.2	-
		-	18.5	-
		8.5*	* 8.3	0.98
		5.6+	+ 10.3	1.84
13.	(1)	4.6*	* 4.5	0.98, 0.83
		5.4*	* 6.5	1.41, 1.20
14.	(1)	-	5.9	-
		-	9.5	-
		-	9.5	-
15.	(1)	11.8*	* 10.9	0.92
	(2)	8.6*	* 8.4	0.98
		2.6+	+ 10.7	4.11
16.	(1)	4.1*	* 3.7	0.90, 0.38
		9.7*		
		5.8+	+ 7.7	1.33
	(2)	17.4*	* 17.8	1.02
			* 12.6	0.72
		12.9	+ 12.3	0.95
18.	(1)	no marker movement observed		

*,+ denote the tracheal transport rates within the experiment corresponding to a pair for comparison. These rates were paired if they were measured when the reference marker and talc were moving simultaneously along a similar segment of trachea. In dog 14, only movement of talc was noted and no comparisons were obtained. In dog 18, no movement of either reference marker or talc was noted, and the experiment was not repeated. The total number of comparisons obtained was 15.

TABLE X

CANINE TRACHEAL MUCOUS TRANSPORT RATES

OF REFERENCE MARKER vs ASBESTOS

Dog Number/Experiment No.		Tracheal Transport Rate (mm/min)		
		Reference Marker	Asbestos	Asbestos ÷ Reference Marker
12.	(1)	14.4*	*11.5	0.80
		14.5+	+20.0	1.38,1.00
		20.0+		
		18.2**	**16.7	0.92
		8.7	-	
	(2)	16.8*	*19.5	1.16
			*24.4	1.45
		13.4+	+13.0	0.97
	(3)	3.3*	*10.8	3.27
		15.6+	+20.9	1.34
		2.9**	**22.7	7.83
			**21.9	7.55
		4.5++	++ 4.5	1.00
			++11.3	2.51
			++11.2	2.48
14.	(1)	14.4*	*12.4	0.86
			*13.4	0.93
			*12.6	0.88
		11.7+	+ 9.5	0.81
		7.0**	** 7.4	1.06
			** 6.4	0.91
	(2)	25.9*	*26.1	1.00,1.12
		23.3*	*21.1	0.81,0.91
		17.6+	+15.5	0.88
		25.6	-	-
15.	(1)	12.6*	*12.6	1.00
19.	(1)	10.7*	* 8.7	0.81,1.93,1.64
		4.5*		
		5.3*		
		12.3+	+10.8	0.84
	(2)	2.6*	*16.3	6.27
		-	23.9	-
		8.3	-	-

Continued:

TABLE X continued:

Dog Number/Experiment No.		Tracheal Transport Rate (mm/min)		
		Reference Marker	Asbestos	Asbestos ÷ Reference Marker
20.	(1)	10.2*	*10.9	1.07
		13.8+	+*19.5	1.91,1.41
			+ 9.6	0.70
	(2)	2.1*	*11.0	5.24,0.81
		13.6*	*10.7	5.10,0.79
		13.9	-	
		3.3	-	
	(3)	14.2*	*15.8	1.11
			*15.8	1.11
			*16.9	1.19
21.	(1)	6.9*	* 8.1	1.17
		12.0+	*+10.0	1.45,0.83
	(2)	12.3*	*13.0	1.06,1.37
		9.5*	*11.4	0.93,1.20
		-	8.3	-
22.	(1)	no marker movement observed		
23.	(1)	no marker movement observed		

*, +, **, ++, denote the tracheal transport rates within the experiment corresponding to a pair for comparison. These rates were paired if they were measured when the reference marker and asbestos were moving simultaneously along a similar segment of trachea. In dogs 22 and 23, no movement of either reference marker or asbestos was noted, and the experiment was not repeated in either dog. The total number of comparisons obtained was 49.

TABLE XI

COMPARISON OF CANINE TRACHEAL MUCOUS TRANSPORT RATES

OF REFERENCE MARKER (L)* vs DIFFERENT POLLUTANTS

Series	No. Dogs/** No. Experiments	No. Comparisons Obtained	No. times L is slower/ tied/faster	Test of Porportion (p value)	Tracheal Transport Rate (mm/min) (mean±SD) (L)	Pollutant	Paired t-test (p value)
L vs pollen	6/13	22	13/4/5	<0.025	16.7±6.7	19.3±9.2	<0.05
L vs talc	6/9	15	5/5/5	n.s.	8.1±4.3	9.5±3.9	n.s.
L vs asbestos	8/15	49	27/5/17	n.s.	11.2±6.1	14.1±5.2	n.s.

* Dowex^{R} anion exchange resin particles (1-X-2, 50-100 mesh)
** Some dogs were used more than once

dogs (Table XI). Five dogs were instrumented more than once, with a maximum of 3 instrumentations to deposit the markers in any dog. In 1 dog, no movement of either pollen or reference marker was observed (Table VIII). A total of 22 comparisons of TTR were obtained; pollen moved faster than reference marker in 13 comparisons, was slower in 5, and in 4 comparisons both rates were tied. Test of proportion was significant for pollen moving faster on more occasions than the reference marker ($p < 0.025$).

In comparing talc and asbestos to reference marker, 9 experiments on 6 dogs and 15 experiments on 8 dogs were performed respectively (Table XI). In the experiments comparing talc and asbestos with the reference marker, 1 dog and 2 dogs in each series, respectively, showed no movement of either marker (Table IX and Table X, respectively). Test of proportion in either series was not significant for either pollutant moving faster or slower on more occasions than the reference marker.

Tracheal mucous transport rates obtained in each series are summarized in Table XI. Only the rates of reference marker or pollutant used in comparisons were included in the calculation of the mean and standard deviation of the transport rate stated. Paired t-test comparing tracheal transport rates of pollen to those of the reference marker achieved significance ($p < 0.05$), but was not significant in the other two series.

CHAPTER V DISCUSSION AND CONCLUSIONS

The two interrelated objectives of this study were to examine the variability of canine mucous transport rates, and to compare the mucous transport rates of 3 naturally-occurring particulate pollutants.

1. Variability of Mucous Transport

a. Comparison of Studies

Various investigators have alluded to the variability of mucous transport rates in general. Kensler and Battista (1966) (80) studied cat tracheal mucous transport rates with carbonlycopodium spore markers *in vitro* and found values between 30 to 45 mm/min, while Carson and Goldhamer (1966) (30) recorded values ranging from 0.25 mm to 25 mm/min *in vivo* in the same species. Sakakura and Proctor (1972) (142), using resin bead markers similar to those used in this study, found *in vivo* canine tracheal transport rates ranging from 0.7 to 35.1 mm/min in 103 measurements in 19 dogs. They found that 1 in 10 dogs had rates below 3 mm/min and 1 in 30 above 30 mm/min. They also found that daily changes in transport rates (observed in 5 dogs measured 3 or more times at weekly intervals) varied by 220% to 450%, and coefficients of variation ranged from 14% to 64% (mean 42%). Sackner et al (1973) (140), using teflon disc markers in a study of 59 dogs, found a maximum tracheal transport rate (mean $\pm$ SD) of 15.6 $\pm$ 9.0 mm/min (coefficient of variation 58%), and the average of the 3 fastest rates (mean $\pm$ SD) 11.8 $\pm$ 7.3 mm/min (coefficient of variation 62%). Chopra et al (1977) (32), using Tc^{99m} or $Indium^{113m}$ labeled albumin microsphere markers found in 10 dogs tracheal mucous transport rates ranging from 9.7 to 29.1 mm/min with a mean $\pm$ SD rate of 19.2 $\pm$ 5.1 mm/min (coefficient of

variation 27%). Yeates et al (1975) (178) using Tc^{99m}-labeled albumin microsphere markers found coefficients of variation of 75% in tracheal mucous transport rates of 42 healthy, non-smoking adults with no difference between sexes. Each individual's short-term coefficient of variation was 25%.

The results of this present study are in agreement in terms of mean tracheal mucous transport rates and coefficients of variation with the results of investigators of canine studies reviewed above, and particularly with the study of Sakakura and Proctor (142) who used similar markers to this present study. It is to be noted that measurements of transport rates in the author's study were obtained during experiments where 2 markers were being tracked simultaneously in the canine trachea (e.g. I^{125}-large and Tc^{99m}-small Dowex^R anion exchange resin particles together). Previous results from this laboratory showed that the presence of different radiolabels together in the trachea (I^{125}, Tc^{99m} or Ga^{67}) did not affect comparison of transport rates, and variability was of the same degree whether a single marker or two markers with different radiolabels were being tracked (36). There was a great degree of variability in canine tracheal mucous transport rates in this study. For example, in a group of 40 dogs, transport rates ranged from 1.8 mm to 25.8 mm/min, with a mean $\pm$ SD of 10.4 $\pm$ 5.3 mm/min (coefficient of variation 51%).

The nature of the 6 markers used to measure mucous transport rates furthermore did not influence its variability, as similar degrees of variability were found with each type of marker. These markers varied from solid resin particles of different sizes and surface

charges to different suspensions (albumin macroaggregates and sulphur colloid). Taking into account results of other investigators who used other types of markers, variability of mucous transport does not appear to be any less with a specific type of marker.

The variability of canine tracheal transport rates of the same individual dogs from day to day was similar to that between different dogs on the same day. The variability of transport rates was no less within a dog in one experimental period than between dogs on the same day, or in the same dog on different days, over a 3-month period. These findings are again in agreement with those of other investigators (140, 142), except one study (Chopra) (32), where it was found that the variation in the same dog was less than that between different dogs. The time between measurements was, however, not stated (32).

b. Factors Influencing Variability

Several factors are generally cited to be responsible for the variability of mucous transport. Genetic factors are likely important. For example, Camner demonstrated that lung clearance rates varied more among unrelated non-smokers than in monozygotic non-smoking twins (28, 29). In addition, the variation of both tracheal (178) and nasal (126) mucous transport rates is less within the same individual than between different individuals. Canine tracheal mucous transport is usually studied in non-homogenous groups of animals which are genetically unrelated (e.g.Appendix A and B). Their constitutional difference may be an important factor in the variability of their tracheal mucous transport rates. It would therefore be of some interest to further study this factor by measuring variability of tracheal mucous transport

rates in a group of dogs from the same litter. The author is unaware of any such previous study.

Mechanical irritation has been shown by Phipps to increase mucous secretion and mucous transport rates in cat trachea (120). Sackner (140) and Friedman (51) both measured canine tracheal transport rates using similar teflon disc markers, but obtained different rates of 15.6 and 7.6 mm/min, respectively. As their methodology was essentially similar, it is likely that the faster rate was related to the fiberoptic bronchoscope which was left in place at the glottis in the former study, thereby producing mechanical irritation to the airway.

The ambient conditions may be an important factor. Hirsch found that dryness of ambient air decreased canine tracheal mucous transport (70) though lesser effects were observed by Bridger and Proctor in canine trachea (19) and by Proctor in human nose (126). The degree of hydration of the animal is also important, as Chopra found that acute dehydration decreased canine tracheal mucous transport (33) and Bang and Bang obtained similar findings on nasal mucous transport in dehydrated herring gulls (11) and day-old chicks (12).

Anesthesia influences mucous transport, and unfortunately most in vivo studies of mucous transport have been performed under anesthesia, with few exceptions (67, 89, 98, 178). Bridger and Proctor found no movement of radio-labeled resin beads in tracheas of deeply anesthetized dogs, movement commencing as anesthesia lightened (19). Landa demonstrated depression of sheep tracheal mucous transport rates by 35% for 3 hours following intravenous pentobarbital anesthesia (89). Patrick and Stirling demonstrated depression of tracheal mucous transport

rates by 50% in rats receiving either thiopental or pentobarbital (119). Gaseous anesthetic agents (e.g. halothane) have been shown to depress tracheal mucous transport in dogs by Forbes (49) and in humans by Burton (22). King et al have found following intravenous pentobarbital anesthesia, an increase in mucous elasticity and viscosity associated with a decrease in collection rate and frog palate transportability of canine tracheal mucus (82).

The site of the airway where mucous transport rates are measured may also affect the observation (9,19,32). Asmendsson and Kilburn studied canine airways and found mucous transport rates of 1.6 mm/min in distal bronchii, 4 mm/min in subsegmental bronchii, 8.3 mm/min in lobar bronchii, and 12.6 mm/min in the trachea (9). There is thus an increase in transport velocity from peripheral to central airways. This gradient has also been observed in the trachea itself in anecdotal accounts, velocities increasing from carina to glottis (Bridger and Proctor) (19),(Chopra) (32), and has been documented in this laboratory (unpublished observations). Sackner et al have described spiral patterns of transport of Teflon disc markers (140). This pattern of transport would also add to the variability of measurements.

No marker movement is sometimes observed in experiments on seemingly healthy animals. For example, Sackner et al found no marker movement in the trachea in 28 of 87 dogs studied (140). Sakakura and Proctor reported no marker movement in 20 of 63 experiments on 34 dogs. However, 25% of the animals revealed squamous metaplasia in their tracheas at autopsy (142). In this present study, 1 in 5 to 6 animals

failed to show marker transport on one or more occasions. In some animals, however, marker movement was observed on another experimental day (dogs I and II, Table VI). The explanation for this phenomenon is not known.

c. Implications of Variability on Experimental Studies of Canine Tracheal Mucous Transport

The variability of canine mucous transport rates thus appears to be large, as shown in this study and in other studies. This inherent variability creates difficulty in interpreting the differences observed in mucous transport rates between two markers with different physicochemical properties if they are obtained in different experiments.

The method chosen to overcome this difficulty was that developed previously in this laboratory by Man et al (36). Canine tracheal mucous transport rates of two different types of radio-labeled markers could be measured simultaneously in an animal. As measurements were performed within the same laboratory and physiological conditions of the animal, direct comparison of transport rates of 2 types of markers were obtained under identical experimental conditions. In this case, the reference marker was I^{125}-labeled anion exchange resin particles, and the other a particulate pollutant labeled with Tc^{99m}. In the light of the foregoing discussion, this method of study appeared the most suitable for determining the effect of physicochemical differences of pollutants on their rate of tracheal mucous transport.

2. Canine Tracheal Mucous Transport of Particulate Pollutants

a. Choice of Pollutants and Reference Marker

Three pollutants were studied, one organic (whole ragweed pollen grains) and two inorganic (talc and asbestos particles). They represent one study in each class of organic and inorganic pollutants. Other choices for study might have included silica, corn dust, and mineral dusts. The radio-labeling techniques used in this study may enable future study of these other pollutants.

Dowex^{R} anion exchange resin particles (1-X-2, 500 mesh) were chosen as the reference marker because of familiarity of use in previous experiments, ease and efficiency of labeling, and high affinity of radio-label to marker (36).

b. Labeling of Pollutants

Direct comparison of the transport rates of the pollutants would be more desirable, and attempts were made to label some of the pollutants with I^{125}. However, attempts to label ragweed pollen with I^{125} by 2 methods (63, 103) were unsuccessful for the purposes of this study. Insufficient protein residues (e.g. tyrosine) (103) on pollen coats and similar lack of suitable residues on talc and asbestos particle surfaces for radio-label binding were probable factors that contributed to the failure of these two methods.

Labeling of the 3 pollutants with Tc^{99m} proved possible. The actual mechanism by which Tc^{99m} binds to each of these pollutants is not well defined (166). Pollen grains possess a tough outer coat (exine) over an inner coat (intine) (66). These possess polymerized

carotenoids, carotenoid esters, cellulose and polypeptide nucleophilic groups that may complex with reduced Tc^{99m} cation (166). Talc ($3MgO.\ 4\ SiO_2.\ H_2O$) and asbestos ($3MgO.\ 2\ SiO_2.\ 2\ H_2O$) also possess nucleophilic (-OH) groups that may also accept radio-label. In the method employed, $Tc^{99m}O_4^-$ is reduced by stannous chloride to Tc^{99m} cation, which presumably binds to nucleophilic groups found on the pollen grains (166) and possibly to the nucleophilic (-OH) radicals found on the surface of talc and asbestos particles (134).

Labeling efficiency for pollen was lower than that achieved by Van Houten et al (166). In their experiments, 30-mesh granular tin was added to stannous chloride reducing agent to assist in keeping the solution in reduced form (166). Complex pH adjustments were also performed to achieve maximal labeling efficiencies for Poa praetensis pollen grains (50-90%). The method used in this present study omitted these 2 latter steps for simplicity, and though labeling efficiency was not as high the activity of labeling achieved with each pollutant was sufficient for peak resolution and identification under the experimental conditions. Affinity of binding of Tc^{99m} to pollutant was also adequate with only very small amounts of isotope coming free in each of the last 3 washings. It is therefore unlikely that significant quantities of radioisotope were leached out onto tracheal mucosa during experiments. Furthermore, it was known from previous experiments that such leaching resulted in a "smear" tracing of activity detected on scanning. This effect was not encountered in any experiments performed.

The physical structure of each pollutant was not appreciably altered by the labeling process, as reviewed by light microscopy.

Poa praetensis pollen grains similarly labeled have been examined by phase and light microscopy and allergenic properties of labeled versus unlabeled grains compared by human scratch skin tests. No alteration in morphology or allergenic properties were detected (166).

c. Comparison of Pollutant Tracheal Transport Rates

The distribution and clearance of inhaled pollutants including uranium dioxide (58), fibrous minerals (107) and asbestos (106), and consequences of asbestos inhalation (167) have been studied in mice. Wagner and Skidmore found the pulmonary clearance of crysotile more rapid than that of amosite and crocidolite forms of asbestos (168). However, comparisons of mucociliary transport of different particulate pollutants have not been previously reported. This study represents one first such attempt.

Tracheal mucous transport of teflon disc markers has been shown to decrease during experimental canine asthma, induced in dogs sensitive to Ascaris suum extract (171). Inhaled sensitivity disease to ragweed pollen can occur in dogs, though rare, and its incidence inadequately documented. It manifests as cough, dyspnea, and production of thick mucus (117) with changes in airway conductance (118). Respiratory system conductance was not monitored in this study. However, there was no evidence from either clinical observation or changes in vital signs in any of the experiments to suggest that an asthmatic attack was occurring as a result of ragweed pollen deposition in the airway.

The results of this study showed no difference in mucous transport rates of talc and asbestos particles compared to the

reference marker. Tracheal mucous transport rates of whole ragweed pollen grains were faster than that of reference marker by both test of proportion and paired t-test ($p < 0.025$ and $p < 0.05$, respectively). By inference, ragweed pollen is transported faster by the mucociliary system than talc and asbestos.

This observation cannot be adequately explained within the present knowledge of mucociliary transport. The possibility of interaction between reference marker and pollutant in the trachea enhancing the mucous transport of ragweed pollen, but not talc or asbestos, is unlikely. The influence of radioisotope label on mucous transport is also unlikely to be of significance, as identical markers labeled with I^{125} or Tc^{99m} have not been shown to have different mucous transport rates (36). Therefore, the observed difference between the transport rates of the resin marker and ragweed pollen grains must be related to the differences between these two entities themselves.

It has been proposed as the thesis hypothesis that the differences in physicochemical properties of individual pollutants may influence their interaction with the mucociliary system and be reflected in differences in their mucous transport rates. The precise nature of this interaction is, however, not known though questioned in the past (176). The distribution of pollen grains and resin particles on the mucous layer appears to be on the mucosal surface, as determined by light microscopy in this study. How each influences the properties of the mucous layer and ciliary kinetics, for example, remains to be elucidated. It is unlikely that differences in physical size alone influence their respective transport rates, as it has been

shown that medium (110 ± 30 microns) and small (3 ± 3 microns) sized resin particles are not transported faster than large resin particles (180 ± 50 microns) (36). Other factors such as shape and density of a particle (156) and its "miscibility" with mucus may also play a part. The chief difficulty in characterizing the physicochemical properties that affect mucous transport of a pollutant in a defined way lies in the lack of adequate choices of markers with well-defined physicochemical properties. It is likely that the effect of the physicochemical properties of a particle on its rate of mucous transport is multifactorial.

3. Conclusions

1. There is a high degree of variability in canine mucous transport rates, as measured and documented in this study and in studies by other investigators.

2. This variability was independent of the type of marker used, and was similar between different dogs as within the same dog. No difference between short-term (one experimental period up to 120 minutes) and long-term (3 months) variation was found.

3. A method enabling simultaneous measurement of canine tracheal mucous transport rates of two different markers was adopted to avoid difficulties caused by this large variability in interpretation of results to allow for comparison of their transport rates. Mucous transport rates of 3 naturally-occurring particulate pollutants were compared.

4. No difference was found between tracheal transport rates of talc or asbestos particles and the reference marker of resin particles. Whole ragweed pollen grain was transported faster than the reference marker, and by inference, talc or asbestos particles. This is consistent with the hypothesis that different naturally-occurring pollutants may interact differently with the mucociliary system with resulting differences in their mucous transport rates.

4. Limitations of This Study

This study examined mucous transport of relatively inert particulate insoluble pollutants. Particulate viable agents such as bacteria and viruses might be handled differently by the mucociliary system. Any inferences of mucous transport of dissimilar pollutants cannot be made from this study.

The amount of particulate pollutant deposited experimentally in this study greatly exceeds the quantity which would be deposited in the trachea under normal physiological conditions. Only very minute quantities of whole pollen grains of various types (diameters 10 to 100 microns) have been demonstrated in tracheobronchial secretions and lung parenchyma in human subjects by Michel et al (105). Airway deposition following nasal inhalation of various commercial forms of asbestos (amosite, crocidolite, and chrysotile) has been examined by Beeckmans (14). Chrysotile is fibrillar and less accessible to distal airways than the other forms of asbestos of similar aerodynamic size (14, 161). This study is therefore not an attempt to study tracheobronchial clearance, because of the large quantities of pollutant used

in the experiments. Rather, it examined one aspect of how the mucociliary system might differ in its transport of different kinds of particulate pollutants using the canine trachea as a model.

Mucous transport is different in the more peripheral airways, with differences in ciliary activity (159), mucus depth and composition (17, 81, 164), and mucous transport rates (9) compared to proximal airways. The precise physiological significance of a quantitative difference of several mm/min in transport rate between two pollutants in the larger airways can only at best be speculative. Interpretations of tracheal mucous transport rates cannot strictly be extended to the peripheral airways. A study of mucous transport in peripheral airways, though perhaps of greater clinical importance, is technically difficult, and the results of clearance studies from distal airways are also influenced by lymphatic clearance and macrophage activity. Mucociliary transport, however, remains an important factor in the retention time of a pollutant in the lung. Lung disease may develop in the presence of competent mucociliary function by dust deposition distal to the mucociliary apparatus. However, decreased mucociliary transport may enhance the pathogenesis of the disease (176).

5. Questions and Recommendations Arising From This Study

1. Minimizing some of the factors that add to the variability of measurements of tracheal mucous transport might allow easier approaches to experimental design and interpretation of data, e.g. minimization of tracheal irritation by instrument design modifications. Measurement of tracheal mucous transport in unanesthetized animals would also be

expected to lessen the degree of variability of observations.

2. More naturally-occurring particulate pollutants need to be similarly investigated. Different classes of pollutants related by certain physical or chemical features, for example, may be studied, and categorized by their mucous transport rates as compared to a reference marker, or even to one another, as "fast" or "slow" transported pollutants. Correlation with the pathophysiological consequences of their inhalation may be attempted.

3. Although the method of radio-labeling by Tc^{99m} has been used successfully in these experiments, more methods need to be developed to radio-label other pollutants for study. Johanson et al have successfully labeled pneumococcal bacteria with Tc^{99m} in lung clearance studies (78). The application to study of mucociliary transport of various bacteria versus a reference marker or a pollutant would be useful. Corn and barley dust have been labeled successfully with Tc^{99m} by the method used in this study (unpublished data).

4. The observed differences in mucous transport rates of various pollutants may be due to differences in transport mechanisms by the mucociliary system. Investigations of these mechanisms would be worthwhile. One approach might be to perform similar studies to this on dogs with an altered mucociliary system, e.g. bronchitic or asthmatic dogs, or in dogs subjected to atropine or vagal blockade. Any changes in the

relationships between "fast" and "slow" transported pollutants in these situations may provide a clue as to the nature of pollutant-mucus interaction.

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APPENDIX A

SELECTION OF DOGS USED FOR STUDY

Strays, donations, abandoned dogs

.....> Edmonton, Red Deer, Wetaskiwin, Leduc Dog Pounds

.....> University of Alberta Dog Farm (in Ellerslie, Alberta) (Healthy, 1-3 year olds only as selected by the resident veterinarian)

.....> Quarantined for a minimum period of 2 weeks, plus vaccinated with DHP (distemper, hepatitis, para-influenza) vaccine and debarked, dewormed, deloused, plus fecal analysis, 4th day post-deworming

.....> To Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta.

APPENDIX B

CHARACTERISTICS OF DOGS USED IN STUDY

All were mongrels (mixed breeds) of both sexes, the predominant mix being listed.

PREDOMINANT MIX	NUMBER USED
Labrador	38
Shepherd	31
Collie	22
Hounds	6
Husky	3
Doberman	2
St. Bernard	2
Chesapeake	2
Boxer	1
Corgi	1
Dalmatian	1
	109

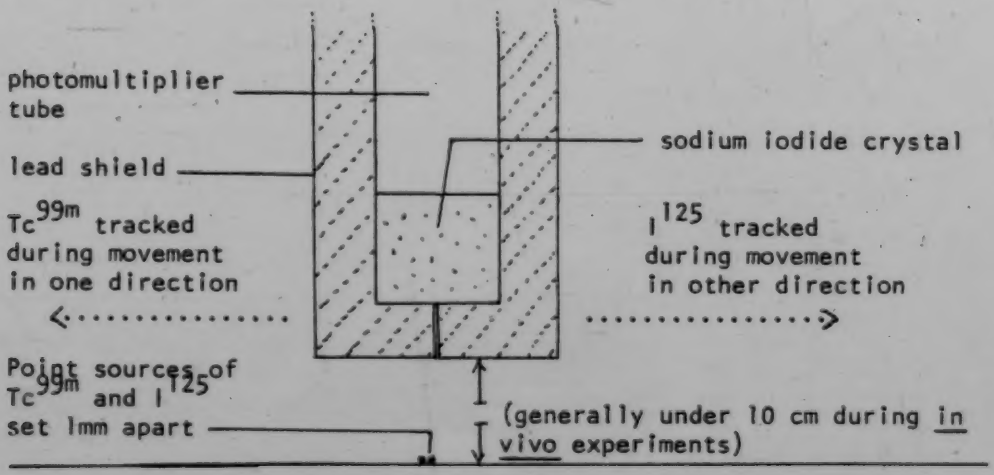
Ages: between 1 - 3 years

Weights: between 12 to 35 kg.

APPENDIX C

DESCRIPTION OF COLLIMATOR AND ISOTOPE DISCRIMINATION

A 3 " by 3" sodium iodide crystal was mounted with lead shielding of 1.5 cm thickness, with a 1 mm slit collimator which travelled orthogonal to the long axis of the trachea during scanning. The *in vitro* arrangement is diagrammed below:


photomultiplier tube
lead shield
Tc^{99m} tracked during movement in one direction
I^{125} tracked during movement in other direction
sodium iodide crystal
Point sources of Tc^{99m} and I^{125} set 1mm apart
(generally under 10 cm during *in vivo* experiments)

Iodine^{125} ($E\gamma \simeq 30$ keV) and Technetium^{99m} ($E\gamma \simeq 140$ keV) were the isotopes used in the study. Preliminary *in vitro* studies with 1 mm point sources of each isotope placed 2 mm apart showed that, after suitable adjustment of upper and lower discrimination settings on the analyzer, Technetium^{99m} cross-over counts in the Iodine^{125} channel were <1% of the gross counts of Iodine^{125}. This allowed successful discrimination of either isotope by this method.

VITA

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University of Singapore, Singapore, 1966-1971

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PUBLICATIONS:

(1) (Abstract)
"Simultaneous Comparison of Canine Mucociliary Tracheal Transport Rates of Dowex® Anion Exchange Particles to Albumin Macroaggregates and Sulphur Colloid", Am Rev Respir Dis [suppl] 119, 226, 1979.